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BIOLOGICAL BULLETIN

ON THE GEOTROPISM OF PARAMECIUM AND SPIROSTOMUM.[1]

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I. Introduction 1
II. The Mechanical Theory 2
1. Experiments on *Paramecium caudatum* 7
2. Experiments on *Paramecium teres* 12
III. The Pressure Theory and Its Criticism 13
IV. The Resistance Theory 14
1. Experiments on *Paramecium caudatum* 19
2. Experiments on *Spirostomum teres* 19
V. The Statocyst Theory and Its Criticism 20
VI. Conclusions 21
VII. Summary 22
VIII. Bibliography 23

I. INTRODUCTION.

Through the kindness of Professor Ralph S. Lillie, I was given an opportunity to study in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., during the summer—July 1–September 4—of 1912. During my stay the following experiments were made under the direction of the instructors in the physiology course, especially of Professor E. P. Lyon. I wish here to acknowledge my indebtedness to Professors Garrey, Knowlton, Lillie, Lyon and Meigs.

The general phenomena of geotropism in the protozoa are well known through the work of Schwarz (22), Aderhold (1), Massart (18), Jensen (11–12), Sosnowski (23), Moore (19), Jennings (5–10), Lyon (16–17), Harper (3–4), and others. The description of the phenomena given in these papers is exhaustive and no recapitulation is needed.

In this paper an attempt has been made to subject to a critical

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

and experimental reëxamination the various theories of geotropism in the protozoa, especially in *Paramecium* and *Spirostomum*. The experimental work has been largely confined, with variations, to the repetition of the work of Lyon and Harper.

As is well known, paramecia are extremely sensitive to chemicals or food, to contact stimuli, to water currents, to mechanical agitation, to changes of temperature, etc. For geotropic experiments, therefore, the sources of those stimuli must be carefully excluded. If this has been done and if paramecia washed in pure distilled water are placed in a vertical *clean* glass tube, they swim upward and gather at the top. In other words, paramecia under proper conditions are negatively geotropic. But Sosnowski (21, p. 131), Platt (20, p. 32), Jennings (9, p. 473; 10, p. 76), Lyon (17, p. 421), Harper (3, p. 995) and others have found considerable variation in the behavior of individuals in different cultures; some orient themselves downward and swim in that direction, while others are apparently indifferent to gravity. Nevertheless, in the majority of cases there is no doubt that gravity is the real "directive force." We are thus confronted by the established fact of negative geotropism in paramecia, but different investigators offer varying explanations for the phenomenon.

II. The Mechanical Theory.

From his study of the reactions of *Euglena* and *Chlamydomonas* to gravity, Schwarz in 1884 reached the conclusion that the negative geotropism of these animals is due to the direct influence of gravity on the organisms, inciting movement in the opposite direction (22). Aderhold in 1888 investigated *Euglena* (1, pp. 317–320) and *Desmid* (1, pp. 339–340), and came to the same conclusion as Schwarz.

Though he seems now to accept the "statolith theory" (25, pp. 526–527), Verworn claimed at first that the effect of the force of gravity exerted upon the protozoa is "purely physical" (24, p. 121). According to him, "the protoplasmic mass of greater specific gravity sinks toward the bottom . . . , while that of less specific gravity rises toward the surface and stays

in that position." On such a "purely physical ground," he criticized Aderhold and believed it self-evident that "in complete quiescence of the flagellum the posterior end of the protist should be directed downward and not the flagellated anterior end." "With its flagellum directed upward and moving it, such an oriented individual must move toward the surface of the water" (24, p. 122). Further, Verworn believed that the force of gravity does not act upon the organism as a stimulus. This view has been called by Davenport the "mechanical theory" (2, p. 121).

After experiments on bacteria, flagellata, and ciliata, Massart in 1891 arrived at the same conclusion as Schwarz and Aderhold; and he stated that his "experiments do not agree with Verworn's theory" (18, p. 166).

After a more extensive and careful study than any of his predecessors had made, Jensen in 1893 "disproved" the mechanical theory of Verworn and proposed his pressure theory instead. But Jensen's experiments were not beyond criticism for his methods were unsatisfactory. He "killed" 50 paramecia in a "filtered alcoholic iodine solution" and watched the individuals fall in the solution to determine which end, anterior or posterior, was directed downward. More than half of them, according to him, sank without any definite orientation of the axis or in "beliebiger Querstellung," while a greater part of the rest sank with their posterior ends directed downward, and the other part with their anterior end directed downward.

From these results and others, he concluded that "the orientation of the *Paramecium* with the long axis vertical and in the direction of gravity would not be brought about by a purely physical effect" (11, p. 455). Jensen also tried similar experiments with *Euglena viridis*, and found that the majority of them sink with their anterior end downward (11, p. 457).

Recently Lyon rightly criticized Jensen's experiments in killing the animals in the alcoholic iodine solution, saying that "the centers of buoyancy and gravity might have been changed through distortion or through localized changes of density in the protoplasm" (17, p. 423). Lyon having questioned Jensen's method, "made use of the fact that the animals when centrifuged

strongly are passively thrown to the bottom of the tube." "A glass tube was drawn into a capillary so fine that paramecia could not turn around in it. The capillary end was dipped into distilled water and the tube filled by capillarity . . . ; then the end was sealed and a drop of water containing paramecia was introduced into the large part of the tube. The tube was fastened to the centrifuge so that the capillary pointed away from the axis of the machine. After strong rotation the capillary was examined under the microscope. The organisms were invariably found with anterior ends directed towards the closed end of the tube." From these results, Lyon concludes: "It is therefore certain that passive paramecia would fall head end down, and that their negative geotropism is the result of an active process on the part of the animals. Jensen's view in this respect is fully confirmed and the mechanical theory is to be laid entirely aside" (17, p. 423).

Judging from Lyon's results, the anterior end seems to be "heavier" in spite of the larger shape of the posterior end. Thus Verworn's theory as well as Davenport's supposition of a static equilibrium (2, p. 122) are found untenable.

Very recently, however, Harper has taken up the problem and in his two articles has again revived the "mechanical theory" (3–4). He adopted the method of Kreidl (13) and that of Prentiss (21) who made crustaceans take in iron-filings as "statoliths" and then applied a magnet. Harper made *Paramecium caudatum* ingest finely divided iron prepared by the alcohol method. In so doing he varied the time for the ingestion of iron, from 30–40 seconds to five minutes. He seems to have obtained his best results when the ingestion of iron was allowed to continue for one minute. "The treated animals," he says in his first article, "swarmed to the top *more quickly* and formed a denser ring there than in the control" (p. 996). From this result and others, Harper drew the conclusion that "the pull of gravity on the *heavier* posterior end may produce a tipping effect which is able to *orient passively but is too weak to stimulate.*"[1] And he believes that here "we have in the normal, quiet, geotropic reactions of *Paramecium* an example of a *purely mechanical* tropism"[1] (3, p. 998).

[1] All italics not in the original.

Harper also claims that the posterior end of normal paramecia is heavier, because they assume the same position in respect to gravity as do those paramecia which have ingested iron in their posterior ends, and which are therefore undoubtedly heavier. But a generalization from such an experiment is not always safe. Spiders, for instance, whose posterior region or abdomen is unmistakably larger and heavier than the anterior region, always orient the head downward *at rest.*[1] Through his experiments, therefore, Harper is hardly justified in drawing his conclusion. Again, according to him, there is no negative geotropism of an active kind on the part of the normal paramecia, though Lyon thinks there is, because the "pull of gravity" "is too weak to stimulate," unless it is augmented by "a greater force" like a strong centrifugal force. "When, however, a stronger force is substituted for gravity so as to produce a sudden orientation, the irritability is affected, and the animal reacts to the change" (3, p. 999).

This conclusion was based on the rheotropism of paramecia. "When . . . the centrifugal effect," he says, "exceeds the pull of gravity and produces too sudden an orientating tendency, this may act as in the ordinary rheotropic reaction against a current. When strongly centrifuged the animal takes a position so that it moves in the water just as the water moves past it in the rheotropic response." "If it allowed itself to be oriented by the centrifugal force with posterior end in advance its relation to the water would be the reverse of what it is in the rheotropic response" (3, p. 998).

From this point of view Harper criticizes Lyon's results. According to the former, "the inference that the anterior end is heavier is contrary to what the shape of the body would indicate, unless the heavier particles are located anteriorly." So he suggests "as an explanation of Lyon's experiment that in strong centrifugalization the same effect is produced at the outset as by mechanical agitation, *i. e.*, the reaction changes to positive." After a repetition of Lyon's centrifugalization experiments,

[1] Moreover, the larvæ of a marine annelid, *Arenicola*, the anterior end of which is larger and heavier than the posterior end, are negatively geotropic. The writer has found by centrifuging that its anterior end is heavier than the posterior end, and further experiments are still in progress (1913).

"the explanation here given" "seemed to him the most satisfactory explanation of the fact that all are found to move with the anterior end outward" (3, p. 998). This suggestion of Harper is worth serious consideration as a criticism of Lyon's experiments, and must not be overlooked; because we know from Sosnowski (23, p. 133) and Moore (19, p. 243) that paramecia which have gathered at the top of a tube often become positively geotropic by a "shock" or "mechanical agitation." The present writer also found this to be true. But two series of questions arise: (*a*) What makes the animals swim downward after the mechanical shock or agitation? If the "pull of gravity" "is too weak to stimulate," it should not produce a positive geotropism any more than a negative one, because the pull of gravity always remains the same before and after the mechanical shock. In Lyon's centrifugalization experiments, however, the "substituted" centrifugal force continued to act until the animals were carried into the capillary tube where they could not turn around. Centrifugalization and temporary mechanical agitation have thus different effects. And (*b*), if the posterior end of the animals is "heavier" than the anterior end, as Harper claims, is it possible that the animals, when *strongly* centrifuged, are really "able to react at the outset of centrifugalization," so as to direct themselves with the "lighter" anterior end away from the axis of the centrifuge? If we believe in a mechanical explanation in this case, as Harper does in the normal case of the "passive orientation," we should hardly expect that this would be the case, because Lyon employed too strong a centrifugal force and the mechanical effect is proportioned to the force acting. It seems to us therefore that the question remains unsettled, though Harper seems satisfied with the mechanical theory.

In his second series of experiments, Harper used a strong electromagnet applied to the iron-ingested paramecia. From the results, he concludes that "the passive sinking of the posterior end is able to orient the animal into a position of gravity equilibrium with the anterior end up" (4, p. 189). As to the magnetic effect, he thinks that it caused in the iron-ingested animals "an upward streaming in the stronger part of the field. Those heavily

loaded animals which move upward in this stream after dispersing above into the weaker part of the magnetic field tend to sink again and cause a return circulation to the bottom. The magnet is effective in producing this circulation by diminishing the effect of gravity on animals containing iron. It also exerts a passive pull upon them and they gradually swing into their finally oriented position in a vertical path under the combined influence of the magnet and gravity" (4, p. 189). Harper could not get any definite results showing a direct effect of the magnet. "The movement toward the magnet," he says, "is the most diffused feature of the circulation, so that superficially it might be set down as the result of the random movements of the animals" (4, p. 185).

1. *Experiments on Paramecium caudatum.*

Experiment a.—Judging from the results of both his experiments, Harper contributes little to the solution of our problem, except his criticism on Lyon's experiments already mentioned. Hence the present writer looked for a method that would obviate Harper's objection to Lyon's conclusions as much as possible. He took advantage of the fact that the paramecia are positively rheotropic, that is, they show the positive reaction to water currents as already stated. For this purpose, he repeated Lyon's method of centrifugalization with a capillary tube like that described by Lyon. A glass tube about 4 mm. in diameter and 7 cm. in length was drawn at the middle into a capillary tube so fine that paramecia (and spirostoma) could not turn around in it.

The parts of *abc* and *cde* were both about 3.5 cm. long. The end of *e* was "sealed" in Lyon's case, but it was *left open* in those used by the writer for the following reasons. The heavier ends of the animals, when centrifuged in the tubes so prepared, are not only passively thrown to the end of the tube away from the axis of the centrifuge so that they close the open end of the tube themselves, but at the same time the current of water is also thrown in the same direction with the animals. The animals being positively rheotropic, as Jennings found (9, pp. 468–473),[1]

[1] The present writer also found that their positive rheotropism is often stronger than their negative geotropism.

they tend naturally to orient themselves in the tube with their anterior end toward the axis of the centrifuge as far as their rheotropism is concerned; thus against the current. Therefore, to some extent, this positive rheotropism, when the animal is centrifuged, will tend to prevent the downward, *i. e.*, positive "orientation" of *Paramecium* to gravity "at the outset of the centrifugalization" as by mechanical agitation. In such a case, if the posterior ends of the animals are "heavier" than their

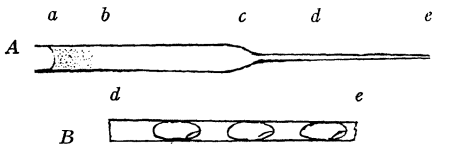


FIG. 1. Tube *A* ready for centrifuge, and *B* a part of capillary after centrifuging, magnified, showing paramecia forced down head first (a slightly modified Lyon's preparation).

anterior ends, as is supposed by Harper, it would be expected that their posterior ends would be passively thrown to the end of the tube *away from* the axis of the centrifuge while the anterior ends would be "oriented" against the water current *toward* the axis. Thus this method obviates in a measure Harper's criticism rendered on Lyon's experiment. Now the writer put some distilled water in the part *bc* of the tube (see diagram), say about 2.5 cm. long, and then added by a small pointed pipette two or three drops of a dense suspension of paramecia, absolutely free from sediment, in the part *ab*. The tube was fastened to the centrifuge as soon as possible, so that the capillary end when centrifuged should be pointed away from the axis of the machine. After centrifuging at a rate of about fifteen revolutions per second for 10 seconds, the capillary was examined under the low power of the microscope. Every animal was without exception found with its anterior end directed toward the open, outer end of the capillary tube in every trial. This was carefully repeated, varying the speed of the centrifuge, that is, with both higher and lower speeds than 15 revolutions per second. The amount of distilled water in the part *bc* was also varied from a column 2.5 cm. long to zero. But this made no difference.

The writer calculates the centrifugal force which he used for the above experiments as follows: The revolutions with the radius about 4 cm. long being about 15 per second, the centrifugal force is given by the formula

$$f = M \cdot \frac{(2\pi r \cdot n)^2}{r},$$

where M = mass in grams, r = radius of circle, n = number of revolutions per second. This gives for $r = 4$ cm. and $n = 15$, $f = M \cdot 35{,}494$ dynes or $M \cdot 36.2$ grams. Moreover, the revolutions were increased to about 20 per second, more or less, as already stated, and then the centrifugal force was about $M \cdot 59{,}101$ dynes or $M \cdot 60.32$ grams. Under these conditions, is it thinkable that such a small animal as *Paramecium* "is able to react at the outset of centrifugalization" against the centrifugal force of the above calculation so as to direct itself with its "heavier" posterior end toward the axis of the centrifuge as Harper claims?

Moreover, Harper states that "an excessive amount of iron may *overload*[1] the animals apparently and caused them to aggregate at the bottom" (3, p. 995). Even though an "overload of iron" might be *quite excessive* for the animal, it could not possibly be comparable with the centrifugal force which the writer used. Nevertheless, Harper thinks that in the case of the *strong* centrifugalization "the animal is able to react" with the supposedly lighter anterior end directed away from the axis of the centrifuge. Such mechanics we can hardly understand.

Experiment b.—With the same point in mind, the writer made another series of experiments with iron-ingested paramecia, as suggested by Harper's experiments. He mixed about a half-gram of finely divided iron prepared by the hydrogen method with about 2 c.c. of a dense culture of paramecia free from sediment. The iron was kept in suspension by drawing the water in and out with a pipette for about 5 minutes. All the paramecia so treated ingested iron in the posterior end of the body. Two or three drops of the culture containing paramecia

[1] Italic not in the original.

so prepared were put in a tube as described in the preceding experiments; the tube was already filled between the points *bc* with about 0.2 c.c. of distilled water. The tube then was centrifuged. The procedure was exactly the same as the preceding experiment. In this case, every animal was found with its posterior end unmistakably directed toward the open outer end of the capillary tube. From the presence of the ingested iron in the posterior end of the animals, we should expect this end to be *heavier* than the anterior end, as Harper says. It is no wonder then that the "heavier posterior end" in this case was passively thrown away from the axis of the centrifuge, as the heavier anterior end was in the normal case. This is the only possible conclusion we can draw. Lyon's view in this respect is fully confirmed, and Harper's explanation is not satisfactory.

Experiment c.—The writer repeated Harper's experiments on the paramecia which had ingested iron as already described. He placed one control tube containing a thick culture of normal paramecia, side by side with a series of five tubes, *a* to *e*, containing iron-ingested animals which were exposed to the iron-containing medium for different periods, as follows: *a* for 30–40 seconds, *b* for two minutes, *c* for 3.30 minutes, *d* for 5 minutes, and *e* for 10 minutes. All of the tubes were in a vertical position. About 18 minutes later, in the control tube, about 70 per cent. of the animals had gathered at the top, 20 per cent. at the bottom, and the rest were scattered through the tube. In all the treated tubes, about 80 per cent. of the animals had gathered at the top and the rest were scattered through the tubes; except in tubes *a* and *b* where there was on the bottom of each tube a small dense gathering which disappeared about 10 minutes later. After about two hours and fifty minutes, in all treated tubes, almost all the animals had gathered at the top, especially in tube *e*, and a very few were scattered here and there, while in the control, 70 per cent. were still at the top and the rest at the bottom.

According to Harper, the ingested iron "particles are at first lodged in the posterior end," and "the changed response is coincident with this condition and tends to disappear as the particles become more evenly distributed through the endo-

plasm" (4, p. 181). The writer continued his observations for about three hours and found that no such change in reaction occurred. At the end of the time, almost all the animals in his case were at the top of the tubes.

About 16 hours later, in the treated tubes, all the paramecia were dead except a few in tubes *a* and *b*. They may have died earlier than this, as the writer did not have a chance to observe them in the meantime. He examined the dead animals under a microscope, but the iron particles were not noticeably "distributed through the endoplasm"; some animals were found vacuolated and many must have broken into pieces, because few dead animals were found. On comparing the above observations with Harper's, we find some differences. According to Harper, "the paramecia rid themselves of the iron after the course of a few hours without apparently harmful effects from a small amount" (3, p. 995). It may be premature to assume a chemical effect on the protoplasm of the iron-ingested paramecia, but we may expect some abnormal reactions of the animals, besides the purely physical; because we saw that all the paramecia treated for 3.5, 5, and 10 minutes died within at least 16 hours, as already stated. In other words, the more iron the animals ingested the quicker their death resulted.

From the above results it is clear that the iron-ingested paramecia are more apt to be negatively geotropic than the normal ones, as Harper has shown. Since the iron is ingested in the posterior region, which presumably is thus made heavier than the anterior region, Harper's view of a "passive orientation" may be true in this particular case. The writer does not mean, however, by "passive orientation," as Harper does, that the "pull of gravity" "is too weak to stimulate" in any case. In point of fact, the normal *Paramecium* orients its anterior end upward not because that end is heavier but *in spite* of it. Of course, therefore, when the posterior end is rendered heavy by iron the orientation is easier. It is here that the "mechanical theory" deserves some credit. It gives us one of the factors of orientation of some animals—like iron-ingested paramecia—against the force of gravity. The "stern-heaviness" or "bow-heaviness," therefore, may be one of the conditions of stimulation

which bring about orientation. But it is not the only condition because, as we have already observed, the negative geotropism of the normal paramecia may be altered by a mechanical agitation to a positive geotropism. We must, therefore, hold with Moore (19, pp. 243–244), Jennings (10, p. 99), and Lyon (17) that there are some additional internal or physiological conditions of orientation in such a case.

Experiment d.—In the experiments on iron-ingested paramecia the writer used an electromagnet not as strong as that used by Harper, employing a binocular microscope placed horizontally for the observations of the movements of the animals, so adjusted that the experimental tube was readily observed. The writer found in these experiments that many animals swam upward, while others swam downward, and a few others toward and away from the electromagnet, which was applied at one side of the tube. According to Harper, the path of paramecia so treated represents the resultant of the pulls of gravity and of the electromagnet. The writer saw some behave in this way, but he could hardly specify it as characteristic. He was unable to detect any orientation with respect to the magnetic force. Possibly the weak electromagnetic force in the writer's case might be the cause of the difference between his results and those of Harper. But even with such a weak force it was observed in many cases that the pull of gravity was easily obscured by the pull of the electromagnet. In a few cases it was observed that the individual when passing in front of the magnet was passively drawn backward toward it when the electric current was connected and moved in a straight line away from it when the current was disconnected. Such cases indicate that the pull of gravity has no effect on the animal in comparison with that of the electromagnet.

2. *Experiments on Spirostomum teres.*

Experiment a.—*Spirostomum teres* is one of the largest of unicellular organisms. The average length of those on which the following experiments were made was about two tenths of a millimeter. Spirostoma from a wild culture were centrifuged to determine their position in a capillary tube in the same way

as in the preceding experiments. The majority of the animals were found with anterior ends directed toward the open outer end of the capillary tube. Owing to the animal's extreme contractility and long form, the writer could not obtain such invariable uniformity of results as he did in the case of *Paramecium*. Nevertheless, the proportion thus directing their anterior end with reference to the centrifugal force was sufficiently great to show that the position was not accidental.

Experiment b.—An attempt was made to repeat the iron-ingestion experiments on spirostoma, but without success. The writer mixed iron filings with about 2 c.c. of the "wild" culture containing a dense suspension of spirostoma in the way that is already described. Within five minutes, all of them broke into pieces beginning at the anterior end and continuing to the posterior, even though none of them ingested iron. Here we see the great injury of iron to the animals; so that the iron ingested by paramecia might not be "harmless" to these animals or at least it might produce some chemical effect on them.

III. The Pressure Theory and its Criticism.

Jensen, having been dissatisfied by Verworn's mechanical theory, proposed the "pressure theory" of geotropism (11, pp. 462–464). According to Jensen, the geotropic orientation of paramecia is determined by the difference in hydrostatic pressure between the upper and lower surfaces of the animal. In the negative geotropism, therefore, the animal moves from the place of high pressure to that of low; in positive geotropism, the animal moves from the place of low pressure to that of high (11, pp. 462–463). Davenport first criticized this theory of Jensen's, and suggested a third theory (2, pp. 122–123). Jennings (9, pp. 473–477) and Lyon (16, pp. xv–xvi) independently disproved Jensen's theory from a physical point of view.

Jennings calculated that "the difference in pressure between the two sides of the organisms is only 1/1,000,000 of the pressure acting everywhere on the surface. Furthermore, Jensen showed that the reaction still occurs when the atmospheric pressure is more than doubled; the effective difference in pressure would be less than 1/2,000,000 of the general pressure" (9, pp. 475–

476). Lyon's calculation was practically the same as Jennings, *i. e.*, 1/1,040,000 (16, p. xv). Since it is well known that the "threshold differential required for the perception of differences in pressure" in man is "about 1/10," both investigators cannot believe that a differential of 1/2,000,000 or 1/1,040,000 is perceptible by paramecia. With regard to the difference in pressure between the anterior and the posterior ends, Jennings thinks that the posterior end being only slightly sensitive to a touch to which the "anterior end reacts violently," "an increase of pressure on the posterior end" "would cause no reaction whatever" (17, p. 476). Neither Jennings nor Lyon calculated the difference in pressure between the anterior and posterior ends of the organism, but this is easily made as follows: The atmospheric pressure being 10,000 mm. of water, and the average length of the paramecia which the writer experimented on about 0.2 mm., the writer calculates the difference in pressure between the anterior and posterior ends of the organisms to be about 1/50,000 of one atmosphere. If "the reaction still occurs when the atmospheric pressure is more than doubled," as Jensen showed, "the effective difference in pressure would then be" about 1/100,000 of one atmosphere. It is hardly conceivable that the difference of either 1/50,000 or 1/100,000 of one atmosphere is perceptible to paramecia. Moreover, Lyon's experiments have shown that "geotropism is intensified in a centrifuge tube which is open away from the axis and in which, therefore, no increase of pressure above atmospheric is possible" (17, p. 431). On the basis of such various evidence, we ought to abandon the pressure theory of geotropism in *Paramecium*.

IV. The Resistance Theory.

A third theory of geotropism, proposed by Davenport, is based on the assumption that the animal "experiences greater resistance (friction + weight) in going upwards even to the slightest extent than in going downward (friction − weight)" (2, p. 122). Lyon calls this the "resistance or weight theory" (17, p. 426). This theory of Davenport was once accepted by Jennings (9, pp. 477–480), though now he rather favors Lyon's theory (10, p. 77) which we shall consider later.

If the weight of organisms or their resistance to the pull of gravity is the only cause of geotropism, negatively geotropic animals should become positively geotropic, and positively geotropic animals should become negatively geotropic in solutions of greater specific gravity than their own, as Platt points out (20, p. 31). Platt at the suggestion of Davenport attempted in vain to solve this problem, because she could not find "paramecia which showed decided geotropic reaction" (20, p. 32). The first approach to this problem was to determine the specific gravity of the *Paramecium*. Jensen first reported it as 1.25 (12, p. 544). He killed *Paramecium aurelia* in solutions of potassium carbonate (12, p. 543) and determined their specific gravity when they were suspended in a solution of known specific gravity. But as Platt has shown (20, p. 34), the specific gravity of the animal when so treated was certainly increased in the solution to some extent. The value of the specific gravity of paramecia obtained by Jensen, therefore, is hardly worth serious consideration. Platt then tried to determine the specific gravity by killing the animals in water to which was "added a few drops of 0.5 per cent. acetic acid." She "also killed paramecia by the fumes of osmic acid." Then she placed the killed paramecia "in a gum-arabic solution of 1.018 specific gravity," whereupon they "remained long suspended in the solution" (20, pp. 35–36). So she thinks that the specific gravity of paramecia so treated "is about 1.017" (20, p. 38). But Platt's method was no better than Jensen's. As she observed that the "reagents caused the paramecia to change their shape somewhat in dying, by becoming wide and shorter," we are not justified in assuming that the specific gravity of the dead animals remained the same. "On the death of the cell," according to Lillie, "there follows a marked and permanent increase in the general permeability, and this change is always associated with a permanent fall in the potential difference between surface and interior" (14, p. 196). Furthermore, as Lyon rightly points out, a viscous gum-arabic solution is likely to hold suspended for a long time even bodies of considerably different density "unless a greater force than gravity be used to separate them from the solution" (17, p. 427). We can therefore place little value on either Jensen's or Platt's results.

Lyon then adopted a method "by which the density of small organisms could be found without any injury or change in them." He "made use of the hæmatocrit attachment of the centrifuge by which very high velocities" (from 10,000 revolutions to 12,000 per minute) "of rotation and consequent high centrifugal force are secured. At the bottom of a *small*[1] tube was placed *a little*[1] gum-arabic solution of known density; above this a *few*[1] drops of water containing paramecia. The tube was then centrifuged about one minute." He thus found that "an average density of paramecia is about 1,048 or 1,049" (17, p. 427).

1. *Experiments on Paramecium caudatum.*

In the results of Jensen, Platt, and Lyon, however, the difference of density is so great that the present writer thought it well to repeat Dr. Lyon's experiments, under his direction, with some modifications. The writer prepared a gum-arabic solution using distilled water. The acid of the solution was carefully neutralized with sodium carbonate. But he regrets that he forgot to dialyze it. He made a series of 40 solutions of different densities from 1.02 to 1.05 in Naples jars. The specific gravity of each was carefully determined by means of a finely graded hydrometer. After some trials the solutions above and below the densities given in the following table were found unnecessary. Then he prepared many small tubes about 12.5 cm. long and 0.4 cm. in diameter, one end of which was sealed. In each trial, two of those tubes were filled about 11 cm. high with gum-arabic solutions of known specific gravity, *e. g.*, the one 1.02 and the other 1.021. This pair was centrifuged to drive the air out. Then on the top of each solution in the two tubes, *one* drop of the culture containing dense paramecia was added by a very small pointed pipette. The tubes so prepared were again centrifuged for one minute with a speed of about 1,300 revolutions per minute. The distribution of the animals in both tubes was then carefully compared. All 40 different solutions having been thus tested, the nearest possible density of the animals was obtained:

[1] Italics not in the original.

I. Tube 1. Density of gum-ar. sol. 1.034, 1,300 revolutions; a few go to bottom.
Tube 2. Density of gum-ar. sol. 1.035, 1,300 revolutions; very few go to bottom.

II. Tube 1. Density of gum-ar. sol. 1.036, 1,300 revolutions; 2 or 3 go to bottom.
Tube 2. Density of gum-ar. sol. 1.037, 1,300 revolutions; none go to bottom.

III. Tube 1. Density of gum-ar. sol. 1.038, 1,300 revolutions; a few go to lower part than middle.
Tube 2. Density of gum-ar. sol. 1.039, 1,300 revolutions; some go to middle.

IV. Tube 1. Density of gum-ar. sol. 1.030, 1,300 revolutions; many go to bottom.
Tube 2. Density of gum-ar. sol. 1.043, 1,300 revolutions; many stay at top.

The writer found thus that the density of the *Paramecium caudatum* is about 1.036 or 1.037. To make sure of the results, three different series of the same experiments were made with different cultures in each series. From two cultures, practically the same results were obtained as the above, but it was found in one culture that the animals when centrifuged were so weak that they were all broken into pieces on the bottom.

In comparison with Lyon's 1.048 or 1.049, we find a difference of about 0.012. Although the difference does not seem very great, it is great for such small organisms. Accordingly at the suggestion of Dr. Knowlton and Dr. Lyon, the writer tested the hydrometer which he used for the measurement of the density of the gum-arabic solutions, by means of a density bottle with Dr. Lyon's help. It was found that the weighing difference between the hydrometer and the bottle was about 0.003. If the test was correct, we may add this difference, 0.003, to 1.036 or 1.037, which makes the density 1.039 or 1.040. If so, the difference between Lyon's 1.048 or 1.049 and the writer's 1.039 or 1.04 becomes a little less.

The only possible source of error in Lyon's experiments is that he may have placed too much "water containing paramecia" above "*a little* gum-arabic solution" in the "*small*" and short tube which he adopted for the hæmatocrit attachment. Under such circumstances, possibly the animals when centrifuged carried some water with them into the gum solution

and lowered the known density of the latter. Nevertheless, there may exist some difference in the specific gravity of the animals in different cultures, as seems to be indicated by the experiments described. This must be borne in mind.

Then the writer prepared a series of 10 tubes about 12.5 cm. long and 0.4 cm. in diameter, in each of which was a definite gum-arabic solution of known density—from 1.034 to 1.044—thoroughly mixed with about two drops or slightly less of the culture containing dense paramecia, filling each up to 12 cm. Three drops of paraffine oil were placed on top of the solution "to exclude oxygen and a possible consequent chemotropic gathering." The writer's observations upon the paramecia so treated agreed with Lyon's in all respects. His words may, therefore, be quoted: "Invariably in all solutions, whether of equal, greater, or less density than the animals, the latter slowly rose and formed a dense ring near the top. The response was slow, as the velocity of swimming in such viscous solutions is extremely small. Two or three hours, or over night, was often necessary. But often the rising ring of animals could be seen in twenty minutes or less. It was typical geotropism very much slowed" (17, p. 428).

The animals so treated lived 5 days in all the solutions above mentioned, though the number of the animals had noticeably decreased at that time. A week after, all the animals in the solutions of densities 1.044 to 1.037 had died, though a few animals in the solutions from 1.036 to 1.034 lived for 13 days. It is rather interesting therefore to note that the animals in the solutions of the greater densities than their own died earlier than the animals in the solutions of the lesser densities. This may not be simply due to density, but density may be at least one of the causes of death of the animals.

To avoid the criticism that the specific gravity of the animals may have been increased by a prolonged stay in such solutions, the writer carefully tested the matter, as Lyon did. After several tests, he found that the animals had increased their density on an average between 0.002 and 0.003 after 13 hours in the solutions. This was determined by centrifuging tubes in which the animals were suspended in solutions of known densities.

The distribution shown by the animals was then compared with the distribution shown in the original determinations of their specific gravity. If the animals in the solution of 1.037 increased their density about 0.003 after 13 hours, they should be distributed in the tube when centrifuged at this time as they had formerly been in the solution of 1.034. The results of Lyon and those of the writer agree fairly with one another in this respect (17, p. 428).

From the results of all the above experiments, we have found that the weight or resistance theory of geotropism is untenable. We have found that no animals which are negatively geotropic become positively geotropic in gum-arabic solutions of greater specific gravity than their own. Thus Lyon's results in all respects are fully confirmed except as regards the exact value of the specific gravity of the animals.

2. *Experiments on Spirostomum teres.*

The same experiments as the above were made with Spirostoma in the same ways. The density of the animals was found:

I.	Tube 1.	Density of gum-ar. sol. 1.022, 1,000 revolutions; some go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.023, 1,000 revolutions; a few go to bottom.
II.	Tube 1.	Density of gum-ar. sol. 1.024, 1,000 revolutions; 4 go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.025, 1,000 revolutions; none go to bottom.
III.	Tube 1.	Density of gum-ar. sol. 1.026, 1,000 revolutions; a few go to lower part.
	Tube 2.	Density of gum-ar. sol. 1.027, 1,000 revolutions; a few go to middle.
IV.	Tube 1.	Density of gum-ar. sol. 1.019, 1,000 revolutions; most go to bottom.
	Tube 2.	Density of gum-ar. sol. 1.030, 1,000 revolutions; most stay near top.

These experiments show that the specific gravity of *Spirostomum teres* lies between 1.024 and 1.025, or very near to 1.025. If we add 0.003 to the 1.025, as we did before, it becomes about 1.028.

Platt obtained a specific gravity of spirostoma of about 1.017 (20, p. 34), which varies by about 0.008 from 1.025. As we have

already pointed out, Platt's method was not as accurate for obtaining the density of the animals as Lyon's. We may therefore consider our results to be the more accurate.

Then nine tubes about 12.5 cm. long and 0.4 cm. in diameter were prepared. They were filled up to about 12 cm. with the solutions of known density—from 1.24 to 1.032—thoroughly mixed with about two drops of the "wild culture" containing dense spirostoma, free from sediment. Three drops of paraffine oil were placed above the solution in each tube.

The negative geotropism of spirostoma is not so marked as usually supposed, and their movements under these conditions were much slower than those of the paramecia in the preceding experiments. About two hours after placing in the solutions of the same density as their own a few rose up to about the middle part of the tubes; a still larger proportion so rose in the solutions of greater densities than their own, but in the solution of 1.024 all were on the bottom, while in that of 1.025 a few rose from the bottom of the tube to the height of 2.5 cm. This suggests that the buoyancy in the solutions of greater densities than their own helped the animals to rise. But about 13 hours later, nearly all the animals in the solutions rose up to near the top. Two weeks later some spirostoma were living in all the tubes except one of 1.027 which was broken 4 days after treatment. At that time the writer had to leave Woods Hole. Meanwhile the number of the animals considerably decreased, but in some tubes a few individuals divided.

V. The Statocyst Theory and Its Criticism.

A fourth theory of geotropism proposed by Lyon is based on the assumption that the *Paramecium* contains protoplasmic materials of different specific gravity. "For internal stimulation the relation of the parts of the cell to each other must be changed in some way by grvaity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position" (17, p. 429). This is called the "statocyst theory" of geotropism in the *Paramecium*. It is worth while to mention, with reference to the general theory of geotropism in animals, that Loeb in 1897 (15, pp. 446–449) had already suggested a similar theory.

Lyon furnishes evidence (though not conclusive) for his theory as follows: "The animals were strongly centrifuged for several minutes in the hæmatocrit attachment. Microscopic examination showed that certain dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal" (17, p. 430).

The writer tried, by means of the centrifuge, to test Lyon's results, but must confess that he could obtain nothing definite. But he thinks now that he ought to have adopted some chemical methods (stains) which might possibly show better results. But since all theories based on the results of external stimulation have been shown above to be fallacious, this theory of Lyon's seems from a physico-chemical point of view to be the most possible and the most reasonable. If this theory is tenable, the matter of the heaviness of either anterior or posterior end is of very little significance in such a unicellular organism as *Paramecium*, because even the lighter end of the organism may possibly contain protoplasmic materials of a specific gravity greater than that of this region taken as a whole.

VI. Conclusions.

So far as the results of our experiments and analyses are concerned, the geotropism of paramecia is due chiefly, if not entirely, to the internal conditions of the organisms rather than to external ones. The results of our experiments have shown that the anterior ends of the animals are heavier than their posterior ends. This fact gives no direct clue to our problem, but furnishes strong evidence against the mechanical, that is, external, theory. On the other hand, if we suppose with Harper that the posterior ends of the animals are "heavier" than their anterior ends, that the "pull of gravity" "is too weak to stimulate the organisms," and that the consequent negative geotropism is due to the orientation of the animals in a "purely mechanical" way, we have no explanation for the lack of uniformity in response to the same stimulus under varying conditions. For instance, when given a "shock," the normal paramecia which form a ring at the top of a tube become positively geotropic and swim

downward, even though the "shock" lasts only a moment. Moreover, as Sosnowski (23, p. 134) and Moore (19, pp. 239–241) have shown, the animals are negatively geotropic at 2° C., while they are positively geotropic at 30° C. As we have already pointed out, certain spiders whose posterior region is much heavier than the anterior, nevertheless orient themselves, when at rest with their head down. Therefore we conclude that without consideration of the internal factors, we have difficulty in explaining the facts, even though we do not absolutely reject the external factors.

A possible method which the writer intends to try later would consist in the following: Immediately after centrifuging a capillary tube containing paramecia, and in which the latter cannot turn around, we may stain them with some dyes and determine the effects of the centrifugal force upon the protoplasmic materials of the organisms. The application of this or similar methods is of the utmost importance for the problem of geotropism not only in paramecia but in animals in general.

VII. Summary.

1. *Paramecium caudatum* and *Spirostomum teres* assume a position with their anterior ends directed away from the axis of the centrifuge. Their anterior ends, therefore, must be heavier than their posterior ends. If so, the negatively geotropic orientation is an active process and the mechanical theory cannot be held.

2. It is not conceivable that the animal could be sensitive to slight differences in pressure such as we have computed must exist between the two sides or the two ends of its body. Therefore the pressure theory is not tenable.

3. *Paramecium* and *Spirostomum* in the gum-arabic solutions of greater, equal, and less specific gravity than their own are still negatively geotropic. Therefore the resistance or weight theory is not correct.

4. The specific gravity of living *Paramecium caudatum* is about 1.037 or 1.037 ± 0.003.

5. The specific gravity of living *Spirostomum teres* is about 1.025 or 1.025 ± 0.003.

6. The statocyst theory of geotropism is the most tenable.

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